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REGγ Mediated Regulation of p21$^{Waf/Cip1}$, p16^{INK4a} and p14^{ARF}/p19^{ARF} *in Vivo*

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ABSTRACT

p21$^{Waf/Cip1}$, p16^{INK4a} and p14^{ARF} (p19^{ARF} in mice) have been demonstrated to be degraded by REGγ-proteasome pathway in an ATP- and ubiquitin-independent manner *in vitro*. However, the *in vivo* roles of REGγ mediated-degradation of p21$^{Waf/Cip1}$, p16^{INK4a} and p14^{ARF} remain unclear. In this study, we showed enhanced expression of p21$^{Waf/Cip1}$, p16^{INK4a} and p19^{ARF} in multiple tissues from REG$\gamma^{-/-}$ mice compared to REG$\gamma^{+/+}$ mice. Furthermore, we examined the expression of p21$^{Waf/Cip1}$, p16^{INK4a} and p14^{ARF} in different cancer tissues and observed that the REGγ protein levels were highly expressed in different human cancers while the level of p21$^{Waf/Cip1}$, p16^{INK4a} and p14^{ARF} appears to be inversely correlated. These results demonstrate that REGγ may exert its function in physiological and pathological conditions through degradation of p21$^{Waf/Cip1}$, p16^{INK4a} and p14^{ARF} *in vivo*.

Keywords: REGγ; p21$^{Waf/Cip1}$; p16^{INK4a}; p14^{ARF}; p19^{ARF}; Cancer

1. Introduction

The INK4a/ARF locus encodes two proteins, p16^{INK4a} and p14^{ARF} (equivalent to p19^{ARF} in mice) [1,2]. Both proteins demonstrate tumor suppressor activity and play an important role in cell proliferation, cell progression and cell growth arrest. Mice lacking either p19^{ARF} or p16^{INK4a} are more prone to tumorigenesis compared to wild-type littermates [3,4]. P16^{INK4a} is a well characterized tumor suppressor and was shown to inhibit the kinase activities of the cyclin D-dependent kinases CDK4 and CDK6 [5,6]. P16^{INK4a} inactivation has been implicated in the deregulation of the cell cycle in the malignant melanoma [7,8].

P14^{ARF} and p19^{ARF} proteins are present in normal cells at low levels but accumulate in response to oncogene activation [9-12]. High p14^{ARF} and 19^{ARF} protein levels promote cell cycle arrest, senescence, or apoptosis by binding directly to the p53-degrading ubiquitin ligase Mdm2 and protecting p53 from Mdm2-mediated degradation [12-14]. The biological properties of p16^{INK4a}, p14^{ARF} and p19^{ARF} have been studied extensively, but the regulation of these proteins remains unknown.

REGγ (also known as PA28γ or PSME3) is a member of the 11S proteasomes which binds and activates the 20S proteasome to promote ATP- and ubiquitin-independent protein degradation. Recent observations show that REGγ could enhance proteasomal degradation of some proteins, such as steroid receptor coactivator-3, p21$^{Waf/Cip1}$ and smurf1 [15-17] and REGγ was also involved in p16^{INK4a} and p19^{ARF} degradation *in vitro* [16]. Furthermore, there is some evidence suggesting that REGγ is involved in cancer progression and REGγ has been reported to be overexpressed in colorectal cancer, thyroid cancer, liver cancer and lung cancer [18-22]. However, there is no evidence about the degradation of p21$^{Waf/Cip1}$, p16^{INK4a}, p14^{ARF}and p19^{ARF} by REGγ *in vivo*.

In this study, we demonstrate that REGγ mediated the degradation of p21$^{Waf/Cip1}$, p16^{INK4a} and p14^{ARF}/p19^{ARF} in

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multiple mouse tissues and in human cancer tissues. In addition, the mRNA levels of p21$^{Waf/Cip1}$, p16^{INK4a} and p19^{ARF} were not affected by REGγ in mouse tissues. Our results provide the first evidence that REGγ plays an important role in the degradation of p21$^{Waf/Cip1}$, p16^{INK4a} and p14^{ARF}/p19^{ARF} *in vivo*.

2. Materials and Methods

2.1. Animals

The REG$\gamma^{-/-}$ mice with C57BL/6 genetic background were acquired from Dr. John J. Monaco and bred in the Animal Core. To generate the mice required in this study, we kept REG$\gamma^{+/-}$ mice intercrosses between males and females. Genotyping of REG$\gamma^{+/+}$ and REG$\gamma^{-/-}$ mice was carried out by PCR analysis of genomic DNA as described.

The REG$\gamma^{+/+}$ and REG$^{-/-}$ mice were born at Mendelian frequency, grew normally and used in the subsequent experiments.

2.2. Antibody and Cell Culture

Antibodies were purchased from Invitrogen (REGγ/PA 28γ), BD Pharmingen (p21$^{Cip/WAF1}$), Sigma (β-actin, p14), Santa Cruz (p16, p19). The HeLa stable cell lines were generated by retroviral shRNA vectors specific for REGγ or a control vector from OriGene (Rockville, MD). These two cell lines were cultured in DMEM supplemented with 10% fetal bovine serum (Gibco).

2.3. RNA Analyses

Tissues were homogenized in 1 ml RNAisoTM Plus (TAKARA). Total RNA was extracted and 2 μg RNA was reversely transcribed into cDNA with M-MLV reverse transcriptase (Invitrogen) following the manufacturer's instruction. The mouse gene-specific primers are as follows:

REGγ sense primer,
5-TCCTCACCAATAGCCACG-3;
REGγ antisense primer,
5-CTCGATCAGCAGCCGAAT-3;
p16 sense primer,
5-GCTGCAGACAGACTGGCCA-3;
p16 antisense primer,
5-GTCCTCGCAGTTCGAATCTG-3;
p19 sense primer,
5-CGCAGGTTCTTGGTCACTGT-3;
p19 antisense primer,
5-TGTTCACGAAAGCCAGAGCG-3;
p21 sense primer,
5-CCTGGTGATGTCCGACCTG-3;
p21 antisense primer,
5-CCATGAGCGCATCGCAATC-3.

2.4. Western Blot Analysis

Tissues were homogenized in liquid nitrogen and lysed using RIPA buffer (1.0 mM EDTA 150 mM 1% Triton X-100 50 mM Tris-HCl (pH 7.4) NaCl 0.1% SDS 1% sodium deoxyholate) for 30 min. Then the homogenized tissues were centrifuged at 12,000 g for 15 min and the supernatants were collected. Equivalent amount of protein from each sample was analyzed by using primary antibodies specific for p16^{INK4a}, p21$^{Waf/Cip1}$, p19^{ARF} and REGγ overnight at 4˚C. After incubation with fluorescent labeled secondary antibody, specific signals for proteins were visualized by LI-COR Odyssey Infrared Imaging System.

2.5. Immunohistochemistry (IHC) and H&E Staining

Tissues were fixed with 4% paraformaldehyde for 48 hours. Then the samples were dehydrated through a graded series of ethanol, embedded in paraffin and sectioned at 4 μm.

Immunohistochemistry (IHC) and H&E staining were performed as described [23]. Antibodies against p16^{INK4a}, p21$^{Waf/Cip1}$, p19^{ARF} and REGγ were used at 1:500, 1:600, 1:600, and 1:400 dilutions, respectively.

2.6. Data Collection and Statistical Analysis

The statistical data was got by GraphPad Prism 5.0 software. The results were expressed as the mean ± s.d. Statistical analysis was performed using the two tailed, paired Student's t test. A p value of less than 0.05 was considered statistically significant.

3. Results

3.1. The p21$^{Waf/Cip1}$, p16^{INK4a}, and p19^{ARF} Expression is Higher in REG$\gamma^{-/-}$ Mouse Tissues

Numerous REGγ targets proteins such as p21$^{Waf/Cip1}$, p16^{INK4a}, and p14^{ARF}/p19^{ARF} (p19^{ARF} in the mouse) of REGγ-proteasome, have been identified *in vitro* [16, 17]. However, the molecular details and *in vivo* biological significance of REGγ, p21$^{Waf/Cip1}$, p16^{INK4a} and p19^{ARF} interplay remain elusive. We investigated cell specific expression patterns of REGγ, p21$^{Waf/Cip1}$, p16^{INK4a} and p19^{ARF} by IHC analysis in cerebellum (**Figure 1(a)**), kidney (**Figure 1(b)**), stomach (**Figure 1(c)**) and liver (**Figure 1(d)**) from REG$\gamma^{+/+}$ and REG$\gamma^{-/-}$ littermate mice. The staining of REGγ was negative in the tissues from REG$\gamma^{-/-}$ mice. Intriguingly, there was an increased expression of p21$^{Waf/Cip1}$, p16^{INK4a} and p19^{ARF} in REGγ depleted tissues, suggesting that REGγ may regulate the expression p21$^{Waf/Cip1}$, p16^{INK4a} and p19^{ARF} *in vivo*.

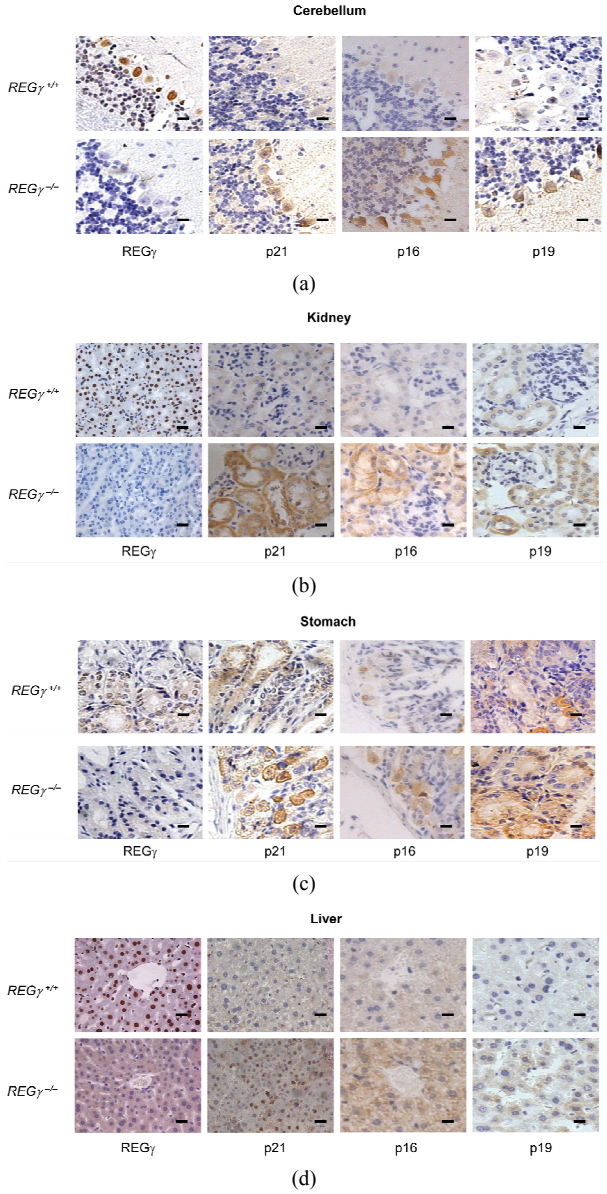

Figure 1. The expression of p21$^{Waf/Cip1}$, p16^{INK4a} and p19^{ARF} is higher in REG$\gamma^{-/-}$ mouse tissues. IHC analysis of REGγ, p21$^{Waf/Cip1}$ p16^{INK4a} and p19ARF in REG$\gamma^{+/+}$ and REG$\gamma^{-/-}$ cerebellum (a); kidney (b); stomach (c) and liver (d) mouse tissues. The result revealed increased p21$^{Waf/Cip1}$ p16^{INK4a} and p19^{ARF} expression in REG$\gamma^{-/-}$ tissues. Scale bar, 100 μm.

3.2. Comparative Analysis of REGγ Protein and mRNA Expression

To verify the REGγ expression observed by IHC, p21$^{Waf/Cip1}$, p16^{INK4a} and p19^{ARF} protein and mRNA levels were examined using extracts from different mouse tissues. Firstly, the relative protein levels were normalized to β-actin and the results showed that p21$^{Waf/Cip1}$, p16^{INK4a} and p19^{ARF} protein levels were higher in REGγ deficient cerebellum (**Figure 2(a)**), stomach (**Figure 2(b)**) and liver (**Figure 2(c)**). The comparison of relative mRNA and protein levels in different tissues may provide intact information about tissue-specific roles for a gene. Next, we consistently observed a higher mRNA expression of p16^{INK4a} and p19^{ARF} in multiple tissues, indicating that REGγ regulated p16^{INK4a} and p19^{ARF} in protein level (**Figure 2(d)**). To further demonstrate that REGγ is involved in p21$^{Waf/Cip1}$, p16^{INK4a} and p19^{ARF} degradation, we generated stable HeLa cell line constitutively expressing either a control non-specific shRNA (shN) or a REGγ-specific shRNA (shR). We monitored the p21$^{Waf/Cip1}$, p16^{INK4a} and p14^{ARF} protein levels in Hela stable cells. As expected, the p21$^{Waf/Cip1}$, p16^{INK4a} and p14ARF protein levels were higher in Hela-ShR cells compared with Hela-shN cells (**Figure 2(e)**). The same phenomenon was also found in Hela cells described previously [16].

3.3. Highly Expressed REGγ and Reduced Expression of p21$^{Waf/Cip1}$, p16^{INK4a} and p14^{ARF} are Observed in Human Cancers

It is known that REGγ is high expressed in lungs, liver, thyroid and colon cancer [20,24]. However, the biological links between REGγ and its correlated genes such as p21$^{Waf/Cip1}$, p16^{INK4a} and p14^{ARF} in cancers remain unknown. Immunohistochemistry (IHC) analysis of REGγ regulated proteins revealed that the REGγ level was high expressed in human cancers such as kidney (**Figure 3(a)**), lungs (**Figure 3(b)**) and brain (**Figure 3(c)**) while the protein level of p21$^{Waf/Cip1}$, p16^{INK4a} and p14^{ARF} was decreased. However, the protein level of p21$^{Waf/Cip1}$, p16^{INK4a} and p14^{ARF} was increased when REGγ level was lower in stomach cancer (**Figure 3(d)**). These results suggest that REGγ mediated degradation of p21$^{Waf/Cip1}$, p16^{INK4a} and p14^{ARF} is one of the important mechanisms by which REGγ mediates its physiological function in different cancers.

4. Discussion

p21$^{Waf/Cip1}$, p19^{ARF} and p16^{INK4a} are important tumor-suppressor proteins. p21$^{Waf/Cip1}$ is encoded by CDKN1A gene, which can bind to and inhibit the activity of cyclin-CDK2 or -CDK4 complexes, and thus functioning as a regulator of cell cycle progression at G1. p16^{INK4a} inhibits Cdk4 and Cdk6 activity leading to activating RB, p19^{ARF} stabilizes p53 protein levels by binding to Mdm2 and promoting its degradation [25]. Mice lacking p21$^{Waf/Cip1}$, p16^{INK4a} or p19^{ARF} are prone to form tumors induced by carcinogens. Currently, several studies showed that REGγ can promote the degradation of p21$^{Waf/Cip1}$ and p53 *in vitro*. However, the detailed link between REGγ and the cyclin-dependent kinase inhibitors is still unclear. This study systematically demonstrated that REGγ can promote the degradation of

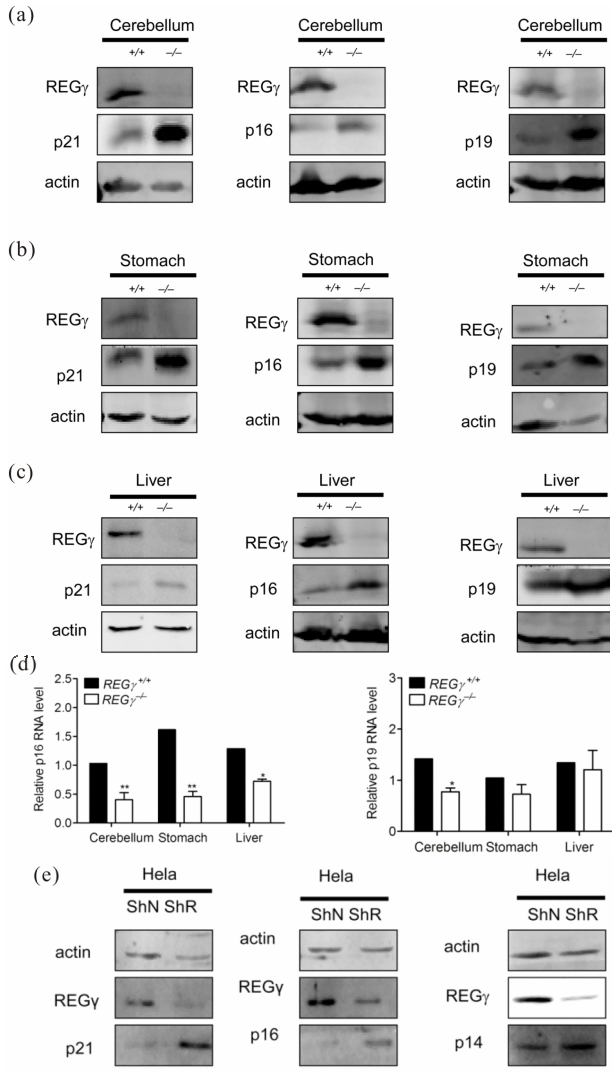

Figure 2. Comparative analysis of REGγ protein and mRNA expression. (a) The protein level of REGγ, p21$^{Waf/Cip1}$, p16^{INK4a} and p19^{ARF} in REG$\gamma^{+/+}$ and REG$\gamma^{-/-}$ mouse cerebellum tissue. Overexpression of p21$^{Waf/Cip1}$ p16^{INK4a} and p19^{ARF} is observed in REG$\gamma^{-/-}$ cerebellum tissue; (b) The protein expression of REGγ, p21$^{Waf/Cip1}$, p16^{INK4a} and p19^{ARF} in REG$\gamma^{+/+}$ and REG$\gamma^{-/-}$ mouse stomach tissue. The expression of p21$^{Waf/Cip1}$, p16^{INK4a} and p19^{ARF} is higher in REG$\gamma^{-/-}$ stomach tissue; (c) The REGγ, p21$^{Waf/Cip1}$, p16^{INK4a} and p19^{ARF} expression of in REG$\gamma^{+/+}$ and REG$\gamma^{-/-}$ mouse liver tissue. The protein level of p21$^{Waf/Cip1}$, p16^{INK4a} and p19^{ARF} is higher in REG$\gamma^{-/-}$ liver tissue; (d) The mRNA levels of p16^{INK4a} and p19^{ARF} in mouse tissues were tested in REG$\gamma^{+/+}$ (n = 3) and REG$\gamma^{-/-}$ (n = 3) mouse tissues. The mRNA levels of p16^{INK4a} and p19^{ARF} is higher in REG$\gamma^{+/+}$ mouse tissues compared with REG$\gamma^{-/-}$ mouse tissues. ($^{*}p < 0.05$; $^{**}p < 0.01$); (e) Expression of REGγ, p21$^{Waf/Cip1}$, p16^{INK4a} and p14^{ARF} in Hela-shN and Hela-shR cell lines.

p21$^{Waf/Cip1}$, p16^{INK4a} and p19^{ARF} *in vivo*.

REGγ-deficient mice [23,26] have been demonstrated growth retardation and cell-specific mitotic defects, indicating a role of REGγ in regulation of cell cycle and cell proliferation. It is well known that p16^{INK4a}, p19^{ARF}, smARF and p21$^{Waf/Cip1}$ are important CDK inhibitors. Therefore, we used the REGγ-knockout mice to detect the effects of REGγ on p16^{INK4a}/p19^{ARF}/p21$^{Waf/Cip1}$ in several normal tissues. As expected, our IHC and blot results showed that the levels of p16^{INK4a}/p19^{ARF}/p21$^{Waf/Cip1}$ were markedly increased in cerebellum, stomach, kidney and liver when REGγ was deleted. Our result demonstrated that REGγ can promote the degradation of CDK inhibitors in normal tissues. These results indicated that REGγ plays an important role in regulating the dynamic balance of body such as organizational self-renewal and tissue remodeling. These results also provide the first time to study the effects of REGγ on CDK inhibitors *in vivo*. Recent studies demonstrate that REGγ can promote proteasome-dependent degradation of cyclin-dependent kinase inhibitors p21$^{Waf/Cip1}$, p16^{INK4a} and p19^{ARF}, in a ubiquitin- and ATP-independent manner [16,17]. However, their conclusion is mainly limited to *in vitro* studies. Thus, our finding is consistent with previous suggestion that REGγ is involved in the regulation of cell cycle and cell proliferation.

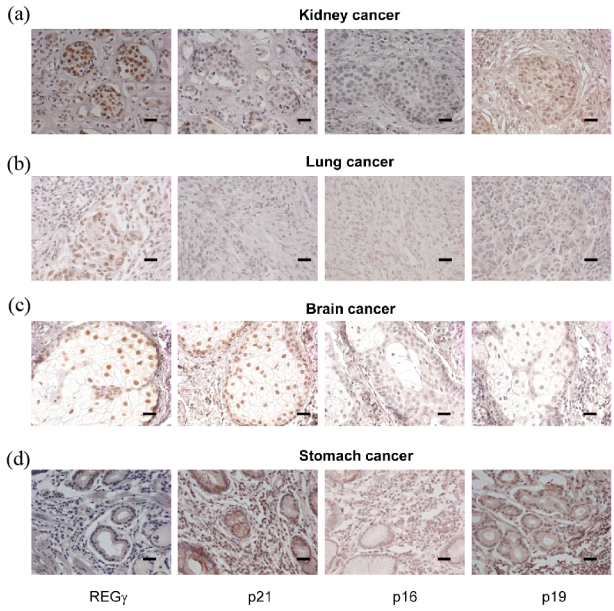

Figure 3. Highly expressed REGγ and decreased REGγ, p21$^{Waf/Cip1}$, p16^{INK4a} and p14^{ARF} protein level are observed in human cancers. IHC analysis of REGγ, p21$^{Waf/Cip1}$, p16^{INK4a} and p14^{ARF} in human kidney cancers (a); lung cancers (b); brain cancers (c) and stomach cancers (d). The result revealed that the REGγ level is high expressed in different human cancers while the protein level of p21$^{Waf/Cip1}$, p16^{INK4a} and p14^{ARF} was decreased. Scale bar, 100 μm.

Besides as the CDK inhibitors, p16^{INK4a}/p19^{ARF}/p21$^{Waf/Cip1}$ are well known as the tumor suppressor. The first genetic evidence supporting a tumor suppressor

activity for p21$^{Waf/Cip1}$ came from the discovery that CDKN1A deficient mice developed spontaneous tumours. Many human cancers such as colorectal, cervical, head and neck, and small-cell lung cancers are associated with reduced p21$^{Waf/Cip1}$ expression. The frequent mutations and deletions of p16^{INK4a} and p14^{ARF} in many types of human cancers cancer cell lines suggested an important role for p16^{INK4a} and p14^{ARF} in carcinogenesis. Thus, the effects of REGγ on p16^{INK4a}/p19^{ARF}/p21$^{Waf/Cip1}$ were investigated in several human cancer tissues. In cancer tissues of stomach, lung, kidney and brain, we found that REGγ has different expression patterns. REGγ's expression was higher in the kidney and lung cancer compared with stomach cancer. These results indicated that REGγ showed its specificity for participating in carcinogensis. Consistently, previous studies revealed that REGγ can degrade not only tumor suppressor but also tumor activator, which suggests its dual function in cancer. Thus, we assumed that the mechanism of REGγ involved in cancer is complicated. Interestingly, we found the levels of p16^{INK4a}/p19^{ARF}/p21$^{Waf/Cip1}$ are firmly regulated by REGγ both in normal tissues and in cancer tissues. When REGγ levels are high in the kidney and lung cancer, the expressions of p16^{INK4a}/p19^{ARF}/p21$^{Waf/Cip1}$ are low. On the contrary, the high expression of p16^{INK4a}/p19^{ARF}/p21$^{Waf/Cip1}$ was observed in the stomach cancer which showed low expression of REGγ. Our results demonstrated that REGγ can promote the degradation of p16^{INK4a}/p19^{ARF}/p21$^{Waf/Cip1}$ not only in normal tissues but also in cancer tissues. REGγ has been reported to be involved in some types of cancer [22,27]. Therefore, we assumed that REGγ maybe play its role in the formation and development of cancer by altering the levels of CDK inhibitors under some conditions.

Recently, REGγ was shown to facilitate the turnover of tumor suppressor p53 by promoting MDM2-mediated p53 ubiquitination [28]. P53 is known as "guardian of the genome" because it shuts down cell division in response to DNA damage. Previous research had linked the function of p14^{ARF} to p53. It is also reported that p53 mediates the DNA damage-induced checkpoint through transactivation of p21$^{Waf/Cip1}$. Thus, the relations between REGγ and p53 *in vivo* are also required to be systemically studied in the future. The members in the CDK inhibitor family also included p15 (INK4B), p18 (INK4C) in INK4 subfamily and p27 (KIP1), p57 (KIP2) in the KIP subfamily. It is still unclear if REGγ can promote the degradation of p15, p18, p27 and p57. Furthermore, the effects of REGγ on p16^{INK4a}/p19^{ARF}/p21$^{Waf/Cip1}$ in the other tissues of mice or in the other types of cancer are needed to be clarified.

In summary, this study is the first to systematically study the effects of REGγ on p16^{INK4a}/p19^{ARF}/p21$^{Waf/Cip1}$ *in vivo*. We found that REGγ can promote the degradation of CDK inhibitor both in normal tissue of mice and in human caner tissues. The data suggest that inhibition of REGγ may be of potential benefit in preventing and treating some types of human cancer.

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REFERENCES

[1] D. E. Quelle, F. Zindy, R. A. Ashmun, *et al.*, "Alternative Reading Frames of the INK4a Tumor Suppressor Gene Encode Two Unrelated Proteins Capable of Inducing Cell Cycle Arrest," *Cell*, Vol. 83, No. 6, 1995, pp. 993-1000. doi:10.1016/0092-8674(95)90214-7

[2] S. W. Lowe and C. J. Sherr, "Tumor Suppression by INK4a-Arf: Progress and Puzzles," *Current Opinion in Genetics & Development*, Vol. 13, No. 1, 2003, pp. 77-83. doi:10.1016/S0959-437X(02)00013-8

[3] M. Serrano, H. Lee, L. Chin, *et al.*, "Role of the INK4a Locus in Tumor Suppression and Cell Mortality," *Cell*, Vol. 85, No. 1, 1996, pp. 27-37. doi:10.1016/S0092-8674(00)81079-X

[4] P. Krimpenfort, K. C. Quon, W. J. Mooi, *et al.*, "Loss of p16INK4a Confers Susceptibility to Metastatic Melanoma in Mice," *Nature*, Vol. 413, No. 6851, 2001, pp. 83-86. doi:10.1038/35092584

[5] M. Serrano, G. J. Hannon and D. Beach, "A New Regulatory Motif in Cell-Cycle Control Causing Specific Inhibition of Cyclin D/CDK4," *Nature*, Vol. 366, No. 6456, 1993, pp. 704-707. doi:10.1038/366704a0

[6] C. J. Sherr, "Principles of Tumor Suppression," *Cell*, Vol. 116, No. 2, 2004, pp. 235-246. doi:10.1016/S0092-8674(03)01075-4

[7] E. Sharpless and L. Chin, "The INK4a/ARF Locus and Melanoma," *Oncogene*, Vol. 22, No. 20, 2003, pp. 3092-3098. doi:10.1038/sj.onc.1206461

[8] R. Ben-Saadon, I. Fajerman, T. Ziv, *et al.*, "The Tumor Suppressor Protein p16^{INK4a} and the Human Papillomavirus Oncoprotein-58 E7 Are Naturally Occurring Lysine-Less Proteins That Are Degraded by the Ubiquitin Sys-

tem. Direct Evidence for Ubiquitination at the N-Terminal Residue," *The Journal of Biological Chemistry*, Vol. 279, No. 40, 2004, pp. 41414-41421. doi:10.1074/jbc.M407201200

[9] S. Bates, A. C. Phillips, P. A. Clark, *et al.*, "p14^{ARF} Links the Tumour Suppressors RB and p53," *Nature*, Vol. 395, No. 6698, 1998, pp. 124-125. doi:10.1038/25867

[10] I. Palmero, C. Pantoja and M. Serrano, "p19ARF Links the Tumour Suppressor p53 to Ras," *Nature*, Vol. 395, No. 6698, 1998, pp. 125-126. doi:10.1038/25870

[11] A. Radfar, I. Unnikrishnan, H. W. Lee, *et al.*, "p19^{ARF} Induces p53-Dependent Apoptosis during Abelson Virus-Mediated Pre-B Cell Transformation," *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 95, No. 22, 1998, pp. 13194-13199. doi:10.1073/pnas.95.22.13194

[12] F. Zindy, R. T. Williams, T. A. Baudino, *et al.*, "ARF Tumor Suppressor Promoter Monitors Latent Oncogenic Signals *in Vivo*," *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 100, No. 26, 2003, pp. 15930-15935. doi:10.1073/pnas.2536808100

[13] R. Honda, and H. Yasuda, "Association of p19^{ARF} with Mdm2 Inhibits Ubiquitin Ligase Activity of Mdm2 for Tumor Suppressor p53," *The EMBO Journal*, Vol. 18, No. 1, 1999, pp. 22-27. doi:10.1093/emboj/18.1.22

[14] C. A. Midgley, J. M. Desterro, M. K. Saville, *et al.*, "An N-Terminal p14 ARF Peptide Blocks Mdm2-Dependent Ubiquitination *in Vitro* and Can Activate p53 *in Vivo*," *Oncogene*, Vol. 19, No. 19, 2000, pp. 2312-2323.

[15] X. Li, D. M. Lonard, S. Y. Jung, *et al.*, "The SRC-3/AIB1 Coactivator Is Degraded in a Ubiquitin- and ATP-Independent Manner by the REGγ Proteasome," *Cell*, Vol. 124, No. 2, 2006, pp. 381-392. doi:10.1016/j.cell.2005.11.037

[16] X. Chen, L. F. Barton, Y. Chi, *et al.*, "Ubiquitin-Independent Degradation of Cell-Cycle Inhibitors by the REGγ Proteasome," *Molecular Cell*, Vol. 26, No. 6, 2007, pp. 843-852. doi:10.1016/j.molcel.2007.05.022

[17] X. Li, L. Amazit, W. Long, *et al.*, "Ubiquitin- and ATP-Independent Proteolytic Turnover of p21 by the REG-gamma-Proteasome Pathway," *Molecular Cell*, Vol. 26, No. 6, 2007, pp. 831-842. doi:10.1016/j.molcel.2007.05.028

[18] T. Okamura, S. Taniguchi, T. Ohkura, *et al.*, "Abnormally High Expression of Proteasome Activator-Gamma in Thyroid Neoplasm," *The Journal of Clinical Endocrinology & Metabolism*, Vol. 88, No. 3, 2003, pp. 1374-1383.

[19] M. Roessler, W. Rollinger, L. Mantovani-Endl, *et al.*, "Identification of PSME3 as a Novel Serum Tumor Marker for Colorectal Cancer by Combining Two-Dimensional Polyacrylamide Gel Electrophoresis with a Strictly Mass Spectrometry-Based Approach for Data Analysis," *Molecular & Cellular Proteomics*, Vol. 5, No. 11, 2006, pp. 2092-2101. doi:10.1074/mcp.M600118-MCP200

[20] Y. Hong, K. S. Ho, K. W. Eu, *et al.*, "A Susceptibility Gene Set for Early Onset Colorectal Cancer That Integrates Diverse Signaling Pathways: Implication for Tumorigenesis," *Clinical Cancer Research*, Vol. 13, No. 4, 2007, pp. 1107-1114. doi:10.1158/1078-0432.CCR-06-1633

[21] H. L. Jia, Q. H. Ye, L. X. Qin, *et al.*, "Gene Expression Profiling Reveals Potential Biomarkers of Human Hepatocellular Carcinoma," *Clinical Cancer Research*, Vol. 13, No. 4, 2007, pp. 1133-1139. doi:10.1158/1078-0432.CCR-06-1025

[22] J. He, L. Cui, Y. Zeng, *et al.*, "REGγ Is Associated with Multiple Oncogenic Pathways in Human Cancers," *BMC Cancer*, Vol. 12, 2012, p. 75. doi:10.1186/1471-2407-12-75

[23] G. Yu, Y. Zhao, J. He, *et al*., "Comparative Analysis of REGγ Expression in Mouse and Human Tissues," *Journal of Molecular Cell Biology*, Vol. 2, No. 4, 2010, pp. 192-198. doi:10.1093/jmcb/mjq009

[24] G. Salvatore, T. C. Nappi, P. Salerno, *et al.*, "A Cell Proliferation and Chromosomal Instability Signature in Anaplastic Thyroid Carcinoma," *Cancer Research*, Vol. 67, No. 21, 2007, pp. 10148-10158. doi:10.1158/0008-5472.CAN-07-1887

[25] Y. Zhang, Y. Xiong and W. G. Yarbrough, "ARF Promotes MDM2 Degradation and Stabilizes p53: ARF-INK4a Locus Deletion Impairs Both the Rb and p53 Tumor Suppression Pathways," *Cell*, Vol. 92, No. 6, 1998, pp. 725-734. doi:10.1016/S0092-8674(00)81401-4

[26] L. F. Barton, H. A. Runnels, T. D. Schell, *et al.*, "Immune Defects in 28-kDa Proteasome Activator Gamma-Deficient Mice," *The Journal of Immunology*, Vol. 172, No. 6, 2004, pp. 3948-3954.

[27] I. Mao, J. Liu, X. Li, *et al.*, "REGγ, a Proteasome Activator and beyond?" *Cellular and Molecular Life Sciences*, Vol. 65, No. 24, 2008, pp. 3971-3980. doi:10.1007/s00018-008-8291-z

[28] Z. Zhang and R. Zhang, "Proteasome Activator PA28 Gamma Regulates p53 by Enhancing Its MDM2-Mediated Degradation," *The EMBO Journal*, Vol. 27, No. 6, 2008, pp. 852-864. doi:10.1038/emboj.2008.25

